KEY WORDS
suspensions; syrup; stability-indicating

REFERENCE
Nahata,M.C.; Morosco,R.S.; Peritore,S.P. Stability of pyrazinamide in two suspensions, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 1558–1560.

SAMPLE
Matrix: urine
Sample preparation: Dilute urine 50-350 times with water, ultrafilter, inject a 20 µL aliquot.

HPLC VARIABLES
Guard column: 11 × 4 5 µm Nucleosil 100 C18
Column: 250 × 4 5 µm Nucleosil 100 C18
Mobile phase: 10 mM KH_2PO_4 adjusted to pH 5.2 with K_2HPO_4
Flow rate: 0.9
Injection volume: 20
Detector: UV

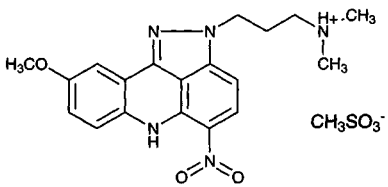
CHROMATOGRAM
Retention time: 26.19
Limit of detection: 300 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
rat; ultrafiltrate

REFERENCE
Mehmedagic,A.; Vérité,P.; Ménager,S.; Tharasse,C.; Chabenat,C.; André,D.; Lafont,O. Determination of pyrazinamide and its main metabolites in rat urine by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *695*, 365–372.

Pyrazoloacridine



Molecular formula: $C_{20}H_{25}N_5O_6S$
Molecular weight: 463.50
CAS Registry No.: 99009-20-8

SAMPLE
Matrix: blood
Sample preparation: Vortex 250 µL plasma with 40 µL 4 µg/mL IS for 10 s, add 600 µL MeOH. Vortex for 20 s, place on ice for 15 min, centrifuge at 1000 g for 10 min, inject a 20-40 µL aliquot of the supernatant.

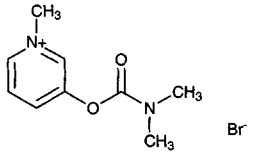
HPLC VARIABLES
Guard column: CN
Column: 150 × 4.6 5 µm Ultrasphere CN (Beckman)
Mobile phase: MeCN:125 mM pH 4.75 ammonium acetate buffer 24:76 (Prepare buffer by dissolving 38.7 g ammonium acetate in 4.0 L water, adjust pH to 4.75 with 25% HCl.)
Flow rate: 0.9
Injection volume: 20-40
Detector: UV 460

CHROMATOGRAM
Retention time: 13.7
Internal standard: ethyl orange (9.5)
Limit of quantitation: 100 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Jayewardene,A.L.; Santoro,J.E.; Gambertoglio,J.G. High-performance liquid chromatographic determination of pyrazoloacridine, a nitro-9-methoxyacridine anticancer agent, in human plasma, *J.Chromatogr.B*, **1997**, *702*, 203–210.

Pyridostigmine bromide



Molecular formula: $C_9H_{13}BrN_2O_2$
Molecular weight: 261.12
CAS Registry No.: 101-26-8
Merck Index: 8161

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 100 µL 500 ng/mL edrophonium in water + 500 µL 100 mM picric acid in 100 mM NaOH (pH adjusted to 7) + 400 µL 100 mM NaH_2PO_4 + 12 mL water saturated dichloromethane, shake vigorously for 5 min, centrifuge at 2000 g for 10 min. Remove 10 mL of the organic phase and add it to 200 µL 1 mM tetrabutylammonium hydrogen sulfate, shake vigorously for 30 s, centrifuge at 2000 g for 2 min, discard most of the organic layer, centrifuge at 2000 g for 1 min, inject a 50 µL aliquot of the aqueous layer. (Store glassware in 100 mM tetramethylammonium chloride solution and wash 5 times with water before use.)

HPLC VARIABLES
Guard column: 50 × 3.2 30-40 µm Perisorb RP-2 (Merck)
Column: 150 × 4.6 5 µm Ultrasphere octyl
Mobile phase: MeCN:water 17:83, pH adjusted to 3 with concentrated sulfuric acid
Flow rate: 2
Injection volume: 50
Detector: UV 214

CHROMATOGRAM
Retention time: 2.5
Internal standard: edrophonium (4.5)
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: neostigmine

KEY WORDS
serum

REFERENCE
De Ruyter,M.G.M.; Cronnelly,R.; Castagnoli,N.,Jr. Reversed-phase, ion-pair liquid chromatography of quaternary ammonium compounds: determination of pyridostigmine, neostigmine and edrophionium in biological fluids, *J.Chromatogr.*, **1980**, *183*, 193–201.

SAMPLE
Matrix: blood

Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH and 5 mL water. 2 mL Plasma + 4 mL 500 mM pH 10.6 phosphate buffer, add to the SPE cartridge, wash with 5 mL 50 mM pH 10.6 phosphate buffer, wash with 5 mL MeOH, elute with 3 mL 1% acetic acid in MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 60°, reconstitute in 60 μL water, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm CPS Hypersil NC-04
Mobile phase: MeCN:buffer 70:30 (Buffer was 0.1% triethylamine in water adjusted to pH 3.2 with acetic acid.)
Column temperature: 22
Flow rate: 1
Injection volume: 50
Detector: UV 272

CHROMATOGRAM
Retention time: 13.5
Limit of detection: 1-2 ng/mL

KEY WORDS
plasma; SPE

REFERENCE
Michaelis,H.C. Determination of pyridostigmine plasma concentrations by high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *534*, 291–294.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a C18 Sep Pak SPE cartridge with 5 mL MeOH and 5 mL water. Adjust the pH of 5 mL urine or plasma to 10.2-10.5 with NaOH, centrifuge for 1 min. Filter (0.45 μm) (urine only) the supernatant and add it to the SPE cartridge, wash with 5 mL water. wash with 5 mL MeOH, push 3 mL air through the SPE cartridge, elute with two 1 mL aliquots of 100 mM acetic acid in MeOH, add 25 μL 25-50 μg/mL methylparaben in water, evaporate the eluate to dryness under nitrogen at 40°, reconstitute in MeOH:water 50:50, inject a 5-100 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:water:acetic acid 20:80:0.5 containing 5 mM 1-octanesulfonic acid (Pic B-8)
Flow rate: 2.5
Injection volume: 5-100
Detector: UV 270

CHROMATOGRAM
Retention time: 3.68
Internal standard: methylparaben (5.93)
Limit of quantitation: 40 ng/mL

OTHER SUBSTANCES
Noninterfering: metabolites

KEY WORDS
SPE; plasma; rat

REFERENCE
Ellin,R.I.; Zvirblis,P.; Wilson,M.R. Method for isolation and determination of pyridostigmine and metabolites in urine and blood, *J.Chromatogr.*, **1982**, *228*, 235–244.

SAMPLE
Matrix: blood, urine

Sample preparation: Plasma. Mix 4 volumes of plasma with 1 volume of 100 mM NaH_2PO_4. 1-2 mL Plasma + 0.5-1 mL buffer, add to a 100 mg Bond Elut CBA cation-exchange SPE cartridge, wash with 5 mL water, wash with 5 mL MeOH, elute with 1 mL acidified MeOH. Evaporate the eluate to dryness under nitrogen at 35°, reconstitute in 120 μL 50 mM sulfuric acid, inject a 100 μL aliquot. Urine. Add 0.2-2 mL urine to a 100 mg Bond Elut CBA cation-exchange SPE cartridge, wash with 5 mL water, wash with 5 mL MeOH, elute with 1.5 mL acidified MeOH. Evaporate the eluate to dryness under nitrogen at 35°, reconstitute in 120 μL 50 mM sulfuric acid, inject a 100 μL aliquot. (Buffer contained 5% sodium carbonate and 5% sodium bicarbonate. Acidified MeOH was 11.4 mL 10.2 M aqueous HCl and 88.6 mL MeOH.)

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:isopropanol:buffer 11.2:1:87.8 (Buffer was 10 mM NaH_2PO_4, 2 mM tetramethylammonium bromide, and 5 mM sodium 1-heptanesulfonate, pH adjusted to 2.8 with sulfuric acid.)
Flow rate: 1
Injection volume: 100
Detector: UV 275

CHROMATOGRAM

Retention time: 8
Limit of detection: 1 ng/mL
Limit of quantitation: 2 ng/mL

KEY WORDS

plasma; SPE; pharmacokinetics

REFERENCE

Biermann,B.; Sommer,N.; Winne,D.; Schumm,F.; Maier,U.; Breyer-Pfaff,U. Renal clearance of pyridostigmine in myasthenic patients and volunteers under the influence of ranitidine and pirenzepine, *Xenobiotica*, **1993**, *23*, 1263–1275.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

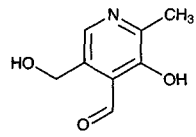
Retention time: 3.228

KEY WORDS

whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Pyridoxal



Molecular formula: $C_8H_9NO_3$
Molecular weight: 167.16
CAS Registry No.: 66-72-8
Merck Index: 8162

SAMPLE
Matrix: baker's yeast, eggs, milk
Sample preparation: Baker's yeast. Mix 50 mg yeast, 50 μL 1 mM isopyridoxal in water, and 3 mL 1 M perchloric acid, vortex for a few s, let stand for 30 min, vortex, centrifuge at 600 g for 15 min. Remove the supernatant, adjust pH to 3-4 with a 10 M KOH, let stand in the refrigerator for a few h. Centrifuge and remove 500 μL of the supernatant and filter (0.45 μm nylon-66) it. Inject a 20 μL aliquot of the filtrate. Egg yolk. Mix 2 g egg yolk with 100 μL 1 mM isopyridoxal water and 6 mL 1 M perchloric acid, vortex for a few s, let stand for 30 min, vortex, centrifuge at 600 g for 15 min. Remove the clear middle part of the extract, adjust pH to 3-4 with a 10 M KOH, let stand in the refrigerator for a few h. Centrifuge and remove 500 μL of the supernatant and filter (0.45 μm nylon-66) it. Inject a 20 μL aliquot of the filtrate. Milk. Mix 2 g egg yolk with 10 μL 1 mM isopyridoxal water and 1 mL 1 M perchloric acid, vortex for a few s, let stand for 30 min, vortex, centrifuge at 600 g for 15 min. Remove the clear middle part of the extract, adjust pH to 3-4 with a 10 M KOH, let stand in the refrigerator for a few h. Centrifuge and remove 500 μL of the supernatant and filter (0.45 μm nylon-66) it. Inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: 30 mm long 5 μm Phenosphere ODS 2 (Phenomenex)
Column: 250 × 4.6 5 μm Phenosphere ODS 2 (Phenomenex)
Mobile phase: 150 mM NaH_2PO_4 adjusted to pH 2.5 with 70% perchloric acid (A 50 mm long column filled with ODS material was placed before the injector.)
Flow rate: 1
Injection volume: 20
Detector: F ex 290 em 389 following post-column derivatization. The column effluent mixed with 1 g/L sodium bisulfite in water pumped at 0.1 mL/min and the mixture flowed through a 2 m × 0.5 mm I.D. PTFE mixing coil to the detector. (The post-column derivatization is only required for pyridoxal phosphate. For the other compounds it is not necessary.)

CHROMATOGRAM
Retention time: 6 (pyridoxal phosphate), 9.5 (pyridoxal)
Internal standard: isopyridoxal (11)

OTHER SUBSTANCES
Extracted: pyridoxamine, pyridoxamine phosphate, 4-pyridoxic acid, 4-pyridoxic acid phosphate, pyridoxine, pyridoxine phosphate

KEY WORDS
post-column reaction

REFERENCE
Argoudelis,C.J. Simple high-performance liquid chromatographic method for the determination of all seven vitamin B6-related compounds, *J.Chromatogr.A*, **1997**, *790*, 83–91.

SAMPLE
Matrix: blood

Sample preparation: 2 mL Serum + 100 μL 1 μM IS + 100 (200 ?) μL 4 M perchloric acid, mix, centrifuge at 1500 g for 5 min. Filter (0.45 μm) the supernatant, inject an aliquot of the filtrate.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Spherisorb ODS2 (12% C, endcapped)
Mobile phase: 67 mM KH_2PO_4 containing 125 μM sodium hexanesulfonate, adjusted to pH 2.5 with concentrated orthophosphoric acid (As column ages it may be necessary to increase concentration of sodium hexanesulfonate to 250 μM to maintain separation.)
Flow rate: 1
Injection volume: 200
Detector: F ex 325 em 400 following post-column reaction. The column effluent mixed with the reagent pumped at 0.5 mL/min and flowed through a 300 mm tube to the detector. (The reagent was 67 mM KH_2PO_4 containing 1 g/L sodium sulfite adjusted to pH 7.5 with Na_2HPO_4.)

CHROMATOGRAM

Retention time: 8.1 (pyridoxal-5'-phosphate), 13.4 (pyridoxal)
Internal standard: pyridoxamine-5'-phosphate (4.6)
Limit of detection: 12.5 nM (pyridoxal), 5 nM (pyridoxal-5'-phosphate)

OTHER SUBSTANCES

Extracted: pyridoxamine, pyridoxine

KEY WORDS

post-column reaction; serum

REFERENCE

Reynolds,T.M.; Brain,A. A simple internally-standardised isocratic HPLC assay for vitamin B_6 in human serum, *J.Liq.Chromatogr.*, **1992**, *15*, 897–914.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 150 μL 3 M perchloric acid, vortex, centrifuge at >1500 g. Remove a 500 μL aliquot of the supernatant and add it to 300 μL buffer, mix, add 100 μL 10 mg/mL acid phosphatase (2 U/mg, grade II, Boehringer-Mannheim) in water, heat at 40° for 16 h, add 150 μL 3 M perchloric acid, vortex, centrifuge at >1500 g, inject a 20 μL aliquot of the supernatant. (Buffer was 1 M sodium acetate/acetic acid containing 24 g/L NaOH, pH 4.6.)

HPLC VARIABLES

Column: 125 × 4 Nucleosil 120 5 C18
Mobile phase: 50 mM Perchloric acid containing 20 mM triethylamine
Flow rate: 2
Injection volume: 20
Detector: F ex 365 em 480 following post-column reaction. The column effluent mixed with the 3.35 g/L semicarbazide hydrochloride in 1.5 M NaOH pumped at 0.5 mL/min and the mixture flowed through a 10 m × 0.3 mm ID crocheted coil of PTFE tubing at 70° to the detector.

CHROMATOGRAM

Retention time: 1.75
Limit of detection: 2 ng/mL

KEY WORDS

post-column reaction; plasma; pharmacokinetics

REFERENCE

Mascher,H. Determination of total pyridoxal in human plasma following oral administration of vitamin B6 by high-performance liquid chromatography with post-column derivatization, *J.Pharm.Sci.*, **1993**, *82*, 972–974.

SAMPLE

Matrix: blood

Sample preparation: 500 μL Plasma + 500 μL 800 mM perchloric acid, vortex vigorously, centrifuge at 35000 g for 5 min, inject a 50-500 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 AQ-302 ODS (YMC)
Mobile phase: 100 mM pH 3.0 KH_2PO_4 containing 100 mM sodium perchlorate and 0.5 g/L sodium bisulfite
Flow rate: 1
Injection volume: 50-500
Detector: F ex 300 em 400

CHROMATOGRAM
Retention time: 3 (pyridoxal phosphate), 5 (pyridoxal)
Limit of detection: 1 pmole (pyridoxal), 0.5 pmole (pyridoxal phosphate)

OTHER SUBSTANCES
Extracted: 4-pyridoxic acid
Simultaneous: pyridoxamine, pyridoxamine phosphate, pyridoxine

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KEY WORDS
plasma; rat; human; protect from light; derivatization

REFERENCE
Kimura,M.; Kanehira,K.; Yokoi,K. Highly sensitive and simple liquid chromatographic determination in plasma of B_6 vitamers, especially pyridoxal 5'-phosphate, *J.Chromatogr.A*, **1996**, *722*, 295–301.

SAMPLE
Matrix: formula, milk
Sample preparation: Mix 8.0 g powdered infant milk with 10 mL water to it. Mix the diluted powder or 10.5 g liquid infant milk with 1 g solid trichloroacetic acid, shake thoroughly with magnetic stirring for 10 min, centrifuge at 1250 g for 10 min, add 3 mL 4% trichloroacetic acid to the solid residue, mix thoroughly for 10 min, centrifuge, discard the solid phase. Combine the two acid extracts and make up to 10 mL with 4% trichloroacetic acid, filter (0.45 μm), inject an aliquot of the filtrate.

HPLC VARIABLES
Guard column: 5 μm Tracer Spherisorb ODS 2 C18 (Teknokroma, Spain)
Column: 250 × 4.6 5 μm Tracer Spherisorb ODS 2 C18 (Teknokroma, Spain)
Mobile phase: MeOH:buffer 15:85 (Buffer was 5 mM octanesulfonic acid and 0.5% triethylamine, pH 3.6.)
Flow rate: 1
Injection volume: 20
Detector: UV 261 for 6 min, UV 287 for 2 min, UV 290 for 5 min, UV 282 for 3 min, UV 268 for 3.5 min, UV 361 for 20.5 min, UV 246 for 20 min

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CHROMATOGRAM
Retention time: 7
Limit of quantitation: ≤50 ng/mL

OTHER SUBSTANCES
Extracted: thiamine, riboflavin, pyridoxine, vitamin B12, niacinamide, folic acid, pyridoxamine

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REFERENCE
Albalá-Hurtado,S.; Veciana-Nogués,M.; Izquierdo-Pulido,M.; Mariné-Font,A. Determination of water-soluble vitamins in infant milk by high-performance liquid chromatography, *J.Chromatogr.A*, **1997**, *778*, 247–253.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 10 μL aliquot of a 1 mg/mL solution in water.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax C8
Mobile phase: 5 mM Sodium perchlorate containing 10 mM sodium 1-hexanesulfonate, pH adjusted to 2.5 with perchloric acid
Column temperature: 45
Flow rate: 0.8
Injection volume: 10
Detector: UV 650 following post-column reaction. The column effluent mixed with 0.5 mg/mL 2,6-dibromo-N-chloro-p-benzoquinoneimine (2,6-dibromoquinone-4-chlorimide) (Eastman) at 1.4 mL/min and flowed through a 2 m × 0.5 mm ID stainless steel coil. The effluent from this coil mixed with 2.5% ammonia solution pumped at 1 mL/min and this mixture flowed through a 2 m × 0.5 mm ID stainless steel coil to the detector.

CHROMATOGRAM
Retention time: 6
Limit of detection: 10 ng

OTHER SUBSTANCES
Simultaneous: pyridoxamine, 4-pyridoxic acid, pyridoxine

KEY WORDS
post-column reaction

REFERENCE
Kawamoto,T.; Okada,E.; Fujita,T. Post-column derivatization of vitamin B6 using 2,6-dibromoquinone-4-chlorimide, *J.Chromatogr.*, **1983**, *267*, 414–419.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Accubond Amino (J & W)
Mobile phase: MeCN:buffer 10 :90 (Buffer was 20 mM phosphoric acid adjusted to pH 3.0 with 20 mM NaOH.)
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 1.4

OTHER SUBSTANCES
Simultaneous: p-aminobenzoic acid, niacinamide, pyridoxamine, pyridoxine, riboflavin, thiamine, vitamin B12

REFERENCE
J & W Catalog, 1992-3, p. 277.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize tissue with 10 volumes 10 mM pH 7.4 potassium phosphate buffer. Remove a 50 μL aliquot and add it to 50 μL 10% trichloroacetic acid, mix, heat at 50° for 15 min, add 35 μL 3.3 M K_2HPO_4, add 1 μL 80 mM KCN, heat at 50° for 25 min, add 12.5 μL 28% phosphoric acid, centrifuge at 13000 g for 10 min, filter (0.45 μm) the supernatant, inject a 5 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 150 × 4 5 μm STR ODS-H (Shimadzu)
Mobile phase: 2 M Acetic acid containing 1 mM sodium 1-heptanesulfonate, adjusted to pH 3.75 with solid KOH
Flow rate: 0.8
Injection volume: 5

Detector: F ex 318 em 418

CHROMATOGRAM
Retention time: 3 (pyridoxal-5-phosphate)
Limit of detection: 50 fmole

KEY WORDS
derivatization; brain

REFERENCE
Naoi,M.; Ichinose,H.; Takahashi,T.; Nagatsu,T. Sensitive assay for determination of pyridoxal-5-phosphate in enzymes using high-performance liquid chromatography after derivatization with cyanide, *J.Chromatogr.*, **1988**, *434*, 209–214.

SAMPLE
Matrix: tissue, CSF, blood
Sample preparation: Dilute rat plasma 1:10. Dilute human plasma 1:25. Homogenize 10 mg liver, 20 mg brain, or 250 μL CSF or diluted plasma with 250 μL 5 or 10% metaphosphoric acid by sonication at 300 W for 30 s, centrifuge at 0-4° at 10000 g for 20 min. Remove the supernatant and add it to 250 μL dichloromethane, vortex, centrifuge at 0-4° at for 15 min, filter (0.22 μm) the aqueous layer, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 3 μm Ultramex C18
Column: 150 × 4.6 3 μm Ultramex C18
Mobile phase: Gradient. A was 33 mM phosphoric acid containing 10 mM 1-octanesulfonic acid adjusted to pH 2.2 with 6 M KOH. B was isopropanol:330 mM phosphoric acid adjusted to pH 2.2 with 6 M KOH. A:B from 100:0 to 0:100 over 10 min, maintain at 0:100 for 15 min, return to initial conditions over 4.5 min, re-equilibrate for 5.5 min. (Every 30 samples flush with water at 0.5 mL/min for 1 h and with isopropanol at 0.2 mL/min for 1 h. Every morning flush with water at 0.5 mL/min for 1 h.)
Flow rate: 1.2
Injection volume: 25
Detector: F ex 328 em 393 following post-column reaction with the reagent. (Reagent was 1 mg/mL sodium bisulfite in 100 mM potassium phosphate buffer adjusted to pH 7.4 with 6 M KOH.)

CHROMATOGRAM
Retention time: 13.6 (pyridoxal), 2.04 (pyridoxal phosphate)
Limit of detection: 4.1 pmole
Limit of quantitation: 10.5 pmole

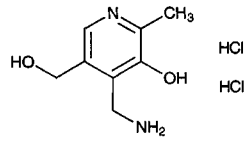
OTHER SUBSTANCES
Extracted: 4-deoxypyridoxine, pyridoxamine, pyridoxamine phosphate, 4-pyridoxic acid, pyridoxine

KEY WORDS
protect from light; rat; human; liver; brain; plasma; CSF; post-column reaction

REFERENCE
Sharma,S.K.; Dakshinamurti,K. Determination of vitamin B_6 vitamers and pyridoxic acid in biological samples, *J.Chromatogr.*, **1992**, *578*, 45–51.

Pyridoxamine dihydrochloride

Molecular formula: $C_8H_{14}Cl_2N_2O_2$
Molecular weight: 241.12
CAS Registry No.: 524-36-7 (di HCl)
Merck Index: 8164



SAMPLE

Matrix: blood
Sample preparation: 2 mL Serum + 100 μL 1 μM IS + 100 (200 ?) μL 4 M perchloric acid, mix, centrifuge at 1500 g for 5 min. Filter (0.45 μm) the supernatant, inject an aliquot of the filtrate.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Spherisorb ODS2 (12% C, endcapped)
Mobile phase: 67 mM KH_2PO_4 containing 125 μM sodium hexanesulfonate, adjusted to pH 2.5 with concentrated orthophosphoric acid (As column ages it may be necessary to increase concentration of sodium hexanesulfonate to 250 μM to maintain separation.)
Flow rate: 1
Injection volume: 200
Detector: F ex 325 em 400 following post-column reaction. The column effluent mixed with the reagent pumped at 0.5 mL/min and flowed through a 300 mm tube to the detector. (The reagent was 67 mM KH_2PO_4 containing 1 g/L sodium sulfite adjusted to pH 7.5 with Na_2HPO_4.)

CHROMATOGRAM

Retention time: 5.5
Internal standard: pyridoxamine-5'-phosphate (4.6)
Limit of detection: 1.5 nM

OTHER SUBSTANCES

Extracted: pyridoxal, pyridoxal-5'-phosphate, pyridoxine

KEY WORDS

post-column reaction; serum

REFERENCE

Reynolds,T.M.; Brain,A. A simple internally-standardised isocratic HPLC assay for vitamin B_6 in human serum, *J.Liq.Chromatogr.*, **1992**, *15*, 897–914.

SAMPLE

Matrix: formulations
Sample preparation: Dilute injections with water, inject a 50 μL aliquot. Dissolve tablets or capsule contents in water (warm if necessary), filter (0.5 μm PTFE), inject a 50 μL aliquot of the filtrate. (Dissolve tablets or other formulations containing proteinaceous material in water at 60°, add 5% trichloroacetic acid (to pH 4.4), filter, inject a 50 μL aliquot.)

HPLC VARIABLES

Guard column: pellicular Corasil
Column: 10 μm μBondapak C18
Mobile phase: Gradient. A was prepared by dissolving 1 g sodium dioctylsulfosuccinate in 170 mL MeOH, add 10 mL concentrated formic acid, make up to 800 mL with water, adjust pH to 2.5 with 1 M KOH, make up to 1 L. B was prepared by dissolving 1 g sodium dioctylsulfosuccinate in 450 mL MeOH, add 10 mL concentrated formic acid, make up to 800 mL with water, adjust pH to 4.6, make up to 1 L. A:B from 100:0 to 0:100 over 25 min (concave curve 9), maintain at 0:100 for 3 min, return to initial conditions over 2 min.
Flow rate: 1.5
Injection volume: 50
Detector: UV 280

CHROMATOGRAM

Retention time: 19

OTHER SUBSTANCES
Simultaneous: folic acid, niacin (UV 254), niacinamide (UV 254), thiamine (UV 254), riboflavin (UV 254), pyridoxine, ascorbic acid

KEY WORDS
injections; capsules; tablets

REFERENCE
Woollard,D.C. New ion-pair reagent for the high-performance liquid chromatographic separation of B-group vitamins in pharmaceuticals, *J.Chromatogr.*, **1984**, *301*, 470–476.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 10 μL aliquot of a 1 mg/mL solution in water.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax C8
Mobile phase: 5 mM Sodium perchlorate containing 10 mM sodium 1-hexanesulfonate, pH adjusted to 2.5 with perchloric acid
Column temperature: 45
Flow rate: 0.8
Injection volume: 10
Detector: UV 650 following post-column reaction. The column effluent mixed with 0.5 mg/mL 2,6-dibromo-N-chloro-p-benzoquinoneimine (2,6-dibromoquinone-4-chlorimide) (Eastman) at 1.4 mL/min and flowed through a 2 m × 0.5 mm ID stainless steel coil. The effluent from this coil mixed with 2.5% ammonia solution pumped at 1 mL/min and this mixture flowed through a 2 m × 0.5 mm ID stainless steel coil to the detector.

CHROMATOGRAM
Retention time: 12
Limit of detection: 10 ng

OTHER SUBSTANCES
Simultaneous: pyridoxal, 4-pyridoxic acid, pyridoxine

KEY WORDS
post-column reaction

REFERENCE
Kawamoto,T.; Okada,E.; Fujita,T. Post-column derivatization of vitamin B6 using 2,6-dibromoquinone-4-chlorimide, *J.Chromatogr.*, **1983**, *267*, 414–419.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Accubond Amino (J & W)
Mobile phase: MeCN:buffer 10 :90 (Buffer was 20 mM phosphoric acid adjusted to pH 3.0 with 20 mM NaOH.)
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 1.3

OTHER SUBSTANCES
Simultaneous: p-aminobenzoic acid, niacinamide, pyridoxal, thiamine, riboflavin, pyridoxine, vitamin B12

REFERENCE
J & W Catalog, 1992-3, p. 277.

SAMPLE

Matrix: tissue, CSF, blood

Sample preparation: Dilute rat plasma 1:10. Dilute human plasma 1:25. Homogenize 10 mg liver, 20 mg brain, or 250 μL CSF or diluted plasma with 250 μL 5 or 10% metaphosphoric acid by sonication at 300 W for 30 s, centrifuge at 0-4° at 10000 g for 20 min. Remove the supernatant and add it to 250 μL dichloromethane, vortex, centrifuge at 0-4° at for 15 min, filter (0.22 μm) the aqueous layer, inject a 25 μL aliquot.

HPLC VARIABLES

Guard column: 30 × 4.6 3 μm Ultramex C18

Column: 150 × 4.6 3 μm Ultramex C18

Mobile phase: Gradient. A was 33 mM phosphoric acid containing 10 mM 1-octanesulfonic acid adjusted to pH 2.2 with 6 M KOH. B was isopropanol:330 mM phosphoric acid adjusted to pH 2.2 with 6 M KOH. A:B from 100:0 to 0:100 over 10 min, maintain at 0:100 for 15 min, return to initial conditions over 4.5 min, re-equilibrate for 5.5 min. (Every 30 samples flush with water at 0.5 mL/min for 1 h and with isopropanol at 0.2 mL/min for 1 h. Every morning flush with water at 0.5 mL/min for 1 h.)

Flow rate: 1.2

Injection volume: 25

Detector: F ex 328 em 393 following post-column reaction with the reagent. (Reagent was 1 mg/mL sodium bisulfite in 100 mM potassium phosphate buffer adjusted to pH 7.4 with 6 M KOH.)

CHROMATOGRAM

Retention time: 25.2 (pyridoxamine), 10 (pyridoxamine phosphate)

Limit of detection: 1.3 pmole

Limit of quantitation: 2.6 pmole

OTHER SUBSTANCES

Extracted: 4-deoxypyridoxine, pyridoxal, pyridoxal phosphate, 4-pyridoxic acid, pyridoxine

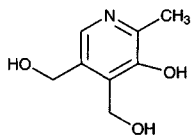
KEY WORDS

protect from light; rat; human; liver; brain; plasma; CSF; post-column reaction

REFERENCE

Sharma,S.K.; Dakshinamurti,K. Determination of vitamin B_6 vitamers and pyridoxic acid in biological samples, *J.Chromatogr.*, **1992**, *578*, 45–51.

Pyridoxine



Molecular formula: $C_8H_{11}NO_3$

Molecular weight: 169.18

CAS Registry No.: 65-23-6, 58-56-0 (HCl)

Merck Index: 8166

SAMPLE

Matrix: baker's yeast, eggs, milk

Sample preparation: Baker's yeast. Mix 50 mg yeast, 50 μL 1 mM isopyridoxal in water, and 3 mL 1 M perchloric acid, vortex for a few s, let stand for 30 min, vortex, centrifuge at 600 g for 15 min. Remove the supernatant, adjust pH to 3-4 with a 10 M KOH, let stand in the refrigerator for a few h. Centrifuge and remove 500 μL of the supernatant and filter (0.45 μm nylon-66) it. Inject a 20 μL aliquot of the filtrate. Egg yolk. Mix 2 g egg yolk with 100 μL 1 mM isopyridoxal water and 6 mL 1 M perchloric acid, vortex for a few s, let stand for 30 min, vortex, centrifuge at 600 g for 15 min. Remove the clear middle part of the extract, adjust pH to 3-4 with a 10 M KOH, let stand in the refrigerator for a few h. Centrifuge and remove 500 μL of the supernatant and filter (0.45 μm nylon-66) it. Inject a 20 μL aliquot of the filtrate. Milk. Mix 2 g egg yolk with 10 μL 1 mM isopyridoxal water and 1 mL 1 M perchloric acid, vortex for a few s, let stand for 30 min, vortex, centrifuge at 600 g for 15 min. Remove the clear middle part of the extract, adjust pH to 3-4 with a 10 M KOH, let stand in the refrigerator

for a few h. Centrifuge and remove 500 μL of the supernatant and filter (0.45 μm nylon-66) it. Inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES

Guard column: 30 mm long 5 μm Phenosphere ODS 2 (Phenomenex)
Column: 250 × 4.6 5 μm Phenosphere ODS 2 (Phenomenex)
Mobile phase: 150 mM NaH_2PO_4 adjusted to pH 2.5 with 70% perchloric acid (A 50 mm long column filled with ODS material was placed before the injector.)
Flow rate: 1
Injection volume: 20
Detector: F ex 290 em 389 following post-column derivatization. The column effluent mixed with 1 g/L sodium bisulfite in water pumped at 0.1 mL/min and the mixture flowed through a 2 m × 0.5 mm I.D. PTFE mixing coil to the detector. (The post-column derivatization is only required for pyridoxal phosphate. For the other compounds it is not necessary.)

CHROMATOGRAM

Retention time: 6.5 (pyridoxine phosphate), 13.5 (pyridoxine)
Internal standard: isopyridoxal (11)

OTHER SUBSTANCES

Extracted: pyridoxal, pyridoxal phosphate, pyridoxamine, pyridoxamine phosphate, 4-pyridoxic acid, 4-pyridoxic acid phosphate

KEY WORDS

post-column reaction

REFERENCE

Argoudelis,C.J. Simple high-performance liquid chromatographic method for the determination of all seven vitamin B6-related compounds, *J.Chromatogr.A*, **1997**, *790*, 83–91.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Serum + 100 μL 1 μM IS + 100 (200 ?) μL 4 M perchloric acid, mix, centrifuge at 1500 g for 5 min. Filter (0.45 μm) the supernatant, inject an aliquot of the filtrate.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Spherisorb ODS2 (12% C, endcapped)
Mobile phase: 67 mM KH_2PO_4 containing 125 μM sodium hexanesulfonate, adjusted to pH 2.5 with concentrated orthophosphoric acid (As column ages it may be necessary to increase concentration of sodium hexanesulfonate to 250 μM to maintain separation.)
Flow rate: 1
Injection volume: 200
Detector: F ex 325 em 400 following post-column reaction. The column effluent mixed with the reagent pumped at 0.5 mL/min and flowed through a 300 mm tube to the detector. (The reagent was 67 mM KH_2PO_4 containing 1 g/L sodium sulfite adjusted to pH 7.5 with Na_2HPO_4.)

CHROMATOGRAM

Retention time: 18.1
Internal standard: pyridoxamine-5'-phosphate (4.6)
Limit of detection: 12.5 nM

OTHER SUBSTANCES

Extracted: pyridoxal, pyridoxal-5'-phosphate, pyridoxamine

KEY WORDS

post-column reaction; serum

REFERENCE

Reynolds,T.M.; Brain,A. A simple internally-standardised isocratic HPLC assay for vitamin B_6 in human serum, *J.Liq.Chromatogr.*, **1992**, *15*, 897–914.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 2.895

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formula, milk
Sample preparation: Mix 8.0 g powdered infant milk with 10 mL water to it. Mix the diluted powder or 10.5 g liquid infant milk with 1 g solid trichloroacetic acid, shake thoroughly with magnetic stirring for 10 min, centrifuge at 1250 g for 10 min, add 3 mL 4% trichloroacetic acid to the solid residue, mix thoroughly for 10 min, centrifuge, discard the solid phase. Combine the two acid extracts and make up to 10 mL with 4% trichloroacetic acid, filter (0.45 μm), inject an aliquot of the filtrate.

HPLC VARIABLES
Guard column: 5 μm Tracer Spherisorb ODS 2 C18 (Teknokroma, Spain)
Column: 250 × 4.6 5 μm Tracer Spherisorb ODS 2 C18 (Teknokroma, Spain)
Mobile phase: MeOH:buffer 15:85 (Buffer was 5 mM octanesulfonic acid and 0.5% triethylamine, pH 3.6.)
Flow rate: 1
Injection volume: 20
Detector: UV 261 for 6 min, UV 287 for 2 min, UV 290 for 5 min, UV 282 for 3 min, UV 268 for 3.5 min, UV 361 for 20.5 min, UV 246 for 20 min

CHROMATOGRAM
Retention time: 10
Limit of quantitation: ≤50 ng/mL

OTHER SUBSTANCES
Extracted: thiamine, riboflavin, vitamin B12, folic acid, niacinamide, pyridoxal, pyridoxamine

REFERENCE
Albalá-Hurtado,S.; Veciana-Nogués,M.; Izquierdo-Pulido,M.; Mariné-Font,A. Determination of water-soluble vitamins in infant milk by high-performance liquid chromatography, *J.Chromatogr.A*, **1997**, *778*, 247–253.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 150 × 6 5 μm Capcell Pak C8 (Shiseido, Japan)
Mobile phase: MeOH:50 mM KH_2PO_4 containing 5 mM tetra-n-butylammonium phosphate 15:85, adjusted to pH 2.6 with 5% orthophosphoric acid (After one week of use, wash the column with water and MeOH:water 70:30 at 1 mL/min for 30 min.)
Column temperature: 30
Flow rate: 1
Injection volume: 10-20
Detector: UV 215

CHROMATOGRAM
Retention time: 3

OTHER SUBSTANCES
Simultaneous: chlorpheniramine, dipotassium glycyrrizate, fumaric acid, m-hydroxybenzoic acid, p-hydroxybenzoic acid, maleic acid, neostigmine methylsulfate, tetrahydrozoline, vitamin B12
Noninterfering: chondroitin sulfate, lysozyme

KEY WORDS
ophthalmic solutions; ion-pair agents

REFERENCE
Yamato,S.; Nakajima,M.; Shimada,K. Simultaneous determination of chlorpheniramine and maleate by high-performance liquid chromatography using tetra-n-butylammonium phosphate as an ion-pair reagent, *J.Chromatogr.A*, **1996**, *731*, 346–350.

SAMPLE
Matrix: formulations
Sample preparation: Pulverize tablets if necessary. Add tablets to 100 mL 5 mM pH 4.5 potassium phosphate buffer, sonicate at 75 W for 2 min, cool to room temperature, make up to 200 mL with buffer, filter (0.45 μm), inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 10 μm LiChrosorb NH2 aminopropyl
Mobile phase: MeCN:5 mM KH_2PO_4 87:13 (Wash column with MeCN:water 10:90 at the end of the day.)
Column temperature: 25
Flow rate: 2
Injection volume: 10
Detector: UV 210

CHROMATOGRAM
Retention time: 2.2

OTHER SUBSTANCES
Simultaneous: pantothenic acid, thiamine, riboflavin, niacinamide

KEY WORDS
tablets

REFERENCE
Hudson,T.J.; Allen,R.J. Determination of pantothenic acid in multivitamin pharmaceutical preparations by reverse-phase high-performance liquid chromatography, *J.Pharm.Sci.*, **1984**, *73*, 113–115.

SAMPLE

Matrix: formulations

Sample preparation: Tablets without iron. Grind 5 tablets to a fine powder, add 10 mL monothioglycerol and 800 mL buffer, sonicate for 30 min, add 150 mL MeOH, make up to 1 L with buffer, filter (GF/C paper), discard first few mL, remove a 10 mL aliquot, make up to 25 mL with mobile phase, inject an aliquot. Tablets with dioctyl sodium sulfosuccinate. Grind 5 tablets to a fine powder, add 10 mL 2-monothioglycerol and 1 g barium chloride, make up to 1 L with buffer, stir vigorously for 30 min, filter (GF/C paper), discard first few mL, inject an aliquot. Capsules with iron. Contents of one capsule + 5 mL 2-monothioglycerol + 2 mL glacial acetic acid + 75 mL buffer, sonicate for 5 min, make up to 100 mL with buffer, stir vigorously for 30 min, filter (GF/C paper), add 300 mg cupferron, stir for 10 min, let stand for 1 h at room temperature, filter (GF/C paper), let stand for 30 min, filter again (if necessary), discard first few mL, inject an aliquot. (Buffer was 48 mL glacial acetic acid and 10 mL triethylamine in 1 L water, adjust pH to 3.6 ± 0.05 with acetic acid or triethylamine, make up to 1.7 L with water.)

HPLC VARIABLES

Column: 100 × 8 Radial Pak A C18 (Waters)

Mobile phase: MeOH:buffer 15:85 (Buffer was 2.20 g sodium heptanesulfonate, 100 mg EDTA, 48 mL glacial acetic acid, and 10 mL triethylamine made up to 1.7 L with water, adjust pH to 3.6 ± 0.05 with acetic acid or triethylamine.)

Flow rate: 2

Injection volume: 10

Detector: UV 280

CHROMATOGRAM

Retention time: 4

OTHER SUBSTANCES

Simultaneous: niacinamide, thiamine, riboflavin, ascorbic acid (UV 254)

KEY WORDS

multi-vitamin; protect from light; tablets; capsules

REFERENCE

Lam,F.-L.; Holcomb,I.J.; Fusari,S.A. Liquid chromatographic assay of ascorbic acid, niacinamide, pyridoxine, thiamine, and riboflavin in multivitamin-mineral preparations, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 1007–1011.

SAMPLE

Matrix: formulations

Sample preparation: Dilute injections with water, inject a 50 μL aliquot. Dissolve tablets or capsule contents in water (warm if necessary), filter (0.5 μm PTFE), inject a 50 μL aliquot of the filtrate. (Dissolve tablets or other formulations containing proteinaceous material in water at 60°, add 5% trichloroacetic acid (to pH 4.4), filter, inject a 50 μL aliquot.)

HPLC VARIABLES

Guard column: pellicular Corasil

Column: 10 μm μBondapak C18

Mobile phase: Gradient. A was prepared by dissolving 1 g sodium dioctylsulfosuccinate in 170 mL MeOH, add 10 mL concentrated formic acid, make up to 800 mL with water, adjust pH to 2.5 with 1 M KOH, make up to 1 L. B was prepared by dissolving 1 g sodium dioctylsulfosuccinate in 450 mL MeOH, add 10 mL concentrated formic acid, make up to 800 mL with water, adjust pH to 4.6, make up to 1 L. A:B 100:0 for 19 min then 0:100 (step gradient) or A:B from 100:0 to 0:100 over 25 min (concave curve 9), maintain at 0:100 for 3 min, return to initial conditions over 2 min.

Flow rate: 1.5

Injection volume: 50

Detector: UV 280

CHROMATOGRAM

Retention time: 17 (step gradient), 16 (curve gradient)

OTHER SUBSTANCES
Simultaneous: folic acid, niacin (UV 254), niacinamide (UV 254), pyridoxamine, thiamine (UV 254), riboflavin (UV 254), ascorbic acid

KEY WORDS
injections; capsules; tablets

REFERENCE
Woollard,D.C. New ion-pair reagent for the high-performance liquid chromatographic separation of B-group vitamins in pharmaceuticals, *J.Chromatogr.*, **1984**, *301*, 470–476.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out 500 mg ground tablets, extract with water, make up to 50 or 100 mL with water, filter, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Nucleosil 10 C18
Mobile phase: MeOH:1% acetic acid 25:75
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 2.8

OTHER SUBSTANCES
Simultaneous: menadione hydrogen sulfite, niacinamide, riboflavin, thiamine
Interfering: ascorbic acid

KEY WORDS
tablets; multi-vitamin

REFERENCE
Sadlej-Sosnowska,N.; Blitek,D.; Wilczynska-Wojtulewicz,I. Determination of menadione sodium hydrogen sulphite and nicotinamide in multivitamin formulations by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *357*, 227–232.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 100 × 4 3 μm Hypersil BDS-C18
Mobile phase: Gradient. MeCN:water adjusted to pH 2.1 from 0.3:99.7 to 25:75 over 11 min
Flow rate: 0.5
Detector: UV 220

CHROMATOGRAM
Retention time: 4.2

OTHER SUBSTANCES
Simultaneous: biotin, caffeine, citric acid, folic acid, niacinamide, niacin, pantothenic acid, riboflavin, saccharin, thiamine, vitamin B12, ascorbic acid

KEY WORDS
tablets

REFERENCE
Hewlett Packard Leaflet 12-5091-7351 EUS, **1993**.

SAMPLE
Matrix: formulations
Sample preparation: Dilute liquid multivitamin formulations, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Lichrosorb RP-8
Mobile phase: Gradient. A was 10 mM KH_2PO_4 containing 5 mM sodium hexanesulfonate adjusted to pH 2.8 with phosphoric acid. B was MeOH. A:B from 90:10 to 71.8:28.2 over 4 min, maintain at 71.8:28.2 for 1.5 min, to 50:50 over 6.5 min, maintain at 50:50 for 5 min, return to initial conditions over 5 min
Flow rate: 1
Injection volume: 5
Detector: UV 290

CHROMATOGRAM
Retention time: 7.65
Internal standard: theobromine (8)
Limit of detection: 0.260 ng

OTHER SUBSTANCES
Simultaneous: folic acid (UV 272), niacin (UV 272), niacinamide (UV 272), thiamine (UV 272), riboflavin (UV 272)

KEY WORDS
liquid multivitamins; degas solutions with helium; protect from light

REFERENCE
Blanco,D.; Sánchez,L.A.; Gutiérrez,M.D. Determination of water soluble vitamins by liquid chromatography with ordinary and narrow-bore columns, *J.Liq.Chromatogr.*, **1994**, *17*, 1525–1539.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with water, filter (0.45 μm), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 100 × 2.1 3 μm Spherisorb ODS-2
Mobile phase: MeOH:buffer:triethylamine 20:80:0.1, pH 2.8 (Buffer was 5 mM sodium hexanesulfonate in 10 mM KH_2PO_4 adjusted to pH 2.8 with phosphoric acid.)
Flow rate: 0.2 for 5 min, to 0.3 over 0.5 min, maintain at 0.3 for 12.5 min
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 3

OTHER SUBSTANCES
Simultaneous: folic acid, niacin (UV 254), riboflavin (UV 254), thiamine (UV 254), niacinamide (UV 254)

KEY WORDS
multivitamin; narrow bore

REFERENCE
Blanco,D.; Sánchez,L.A.; Gutiérrez,M.D. Determination of water soluble vitamins by liquid chromatography with ordinary and narrow-bore columns, *J.Liq.Chromatogr.*, **1994**, *17*, 1525–1539.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 10 μL aliquot of a 1 mg/mL solution in water.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax C8
Mobile phase: 5 mM Sodium perchlorate containing 10 mM sodium 1-hexanesulfonate, pH adjusted to 2.5 with perchloric acid
Column temperature: 45
Flow rate: 0.8
Injection volume: 10
Detector: UV 650 following post-column reaction. The column effluent mixed with 0.5 mg/mL 2,6-dibromo-N-chloro-p-benzoquinoneimine (2,6-dibromoquinone-4-chlorimide) (Eastman) at 1.4 mL/min and flowed through a 2 m × 0.5 mm ID stainless steel coil. The effluent from this coil mixed with 2.5% ammonia solution pumped at 1 mL/min and this mixture flowed through a 2 m × 0.5 mm ID stainless steel coil to the detector.

CHROMATOGRAM
Retention time: 8
Limit of detection: 10 ng

OTHER SUBSTANCES
Simultaneous: pyridoxal, pyridoxamine, 4-pyridoxic acid

KEY WORDS
post-column reaction

REFERENCE
Kawamoto,T.; Okada,E.; Fujita,T. Post-column derivatization of vitamin B6 using 2,6-dibromoquinone-4-chlorimide, *J.Chromatogr.*, **1983**, *267*, 414–419.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Accubond Amino (J & W)
Mobile phase: MeCN:buffer 10 :90 (Buffer was 20 mM phosphoric acid adjusted to pH 3.0 with 20 mM NaOH.)
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 1.4

OTHER SUBSTANCES
Simultaneous: p-aminobenzoic acid, niacinamide, pyridoxal, pyridoxamine, thiamine, riboflavin, vitamin B12

REFERENCE
J & W Catalog, 1992-3, p. 277.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 100 × 4.6 Spheri-5 RP-8
Mobile phase: Gradient. A was 100 mM pH 4.7 acetate buffer. B was MeCN:100 mM pH 4.7 acetate buffer 25:75.
Column temperature: 26
Flow rate: 4
Detector: UV 254

CHROMATOGRAM
Retention time: 1.5

OTHER SUBSTANCES
Simultaneous: niacin, riboflavin, thiamine, niacinamide, ascorbic acid

REFERENCE
Rainin Catalog, C1-94, **1994,** p. 7.21.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 33 × 4.6 3 μm Supelcosil LC-8-DB
Mobile phase: MeOH:buffer 15:85 (Buffer was 4.3 mM sodium hexanesulfonate containing 0.1% triethylamine, adjusted to pH 2.8 with phosphoric acid.)
Column temperature: 35
Flow rate: 1
Detector: UV 200

CHROMATOGRAM
Retention time: 1.1

OTHER SUBSTANCES
Simultaneous: niacin, pantothenic acid, riboflavin, thiamine, niacinamide, ascorbic acid

REFERENCE
Rainin Catalog, C1-94, **1994,** p. 780.

SAMPLE
Matrix: tissue, CSF, blood
Sample preparation: Dilute rat plasma 1:10. Dilute human plasma 1:25. Homogenize 10 mg liver, 20 mg brain, or 250 μL CSF or diluted plasma with 250 μL 5 or 10% metaphosphoric acid by sonication at 300 W for 30 s, centrifuge at 0-4° at 10000 g for 20 min. Remove the supernatant and add it to 250 μL dichloromethane, vortex, centrifuge at 0-4° at for 15 min, filter (0.22 μm) the aqueous layer, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 3 μm Ultramex C18
Column: 150 × 4.6 3 μm Ultramex C18
Mobile phase: Gradient. A was 33 mM phosphoric acid containing 10 mM 1-octanesulfonic acid adjusted to pH 2.2 with 6 M KOH. B was isopropanol:330 mM phosphoric acid adjusted to pH 2.2 with 6 M KOH. A:B from 100:0 to 0:100 over 10 min, maintain at 0:100 for 15 min, return to initial conditions over 4.5 min, re-equilibrate for 5.5 min. (Every 30 samples flush with water at 0.5 mL/min for 1 h and with isopropanol at 0.2 mL/min for 1 h. Every morning flush with water at 0.5 mL/min for 1 h.)
Flow rate: 1.2
Injection volume: 25
Detector: F ex 328 em 393 following post-column reaction with the reagent. (Reagent was 1 mg/mL sodium bisulfite in 100 mM potassium phosphate buffer adjusted to pH 7.4 with 6 M KOH.)

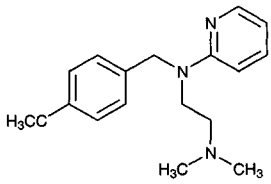
CHROMATOGRAM
Retention time: 18.5
Limit of detection: 2.6 pmole
Limit of quantitation: 7.5 pmole

OTHER SUBSTANCES
Extracted: 4-deoxypyridoxine, pyridoxal, pyridoxal phosphate, pyridoxamine, pyridoxamine phosphate, 4-pyridoxic acid

KEY WORDS
protect from light; rat; human; liver; brain; plasma; CSF; post-column reaction

REFERENCE
Sharma,S.K.; Dakshinamurti,K. Determination of vitamin B_6 vitamers and pyridoxic acid in biological samples, *J.Chromatogr.*, **1992**, *578*, 45–51.

Pyrilamine



Molecular formula: $C_{17}H_{23}N_3O$
Molecular weight: 285.39
CAS Registry No.: 91-84-9, 59-33-6 (maleate)
Merck Index: 8168
Lednicer No.: 1 51

SAMPLE
Matrix: blood
Sample preparation: 4 mL Whole blood + 10 mL pH 10.0 phosphate buffer (μ = 0.4), vortex, add 5 mL chloroform:hexane 40:60, shake gently horizontally for 30 min, centrifuge. Remove 3 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 250 μL dichloromethane, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm Micropak CN-10
Mobile phase: n-Hexane:dichloromethane:MeCN:propylamine 50:25:25:0.1
Column temperature: 30
Flow rate: 2
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 4.0
Internal standard: pyrilamine maleate (mepyramine maleate)

OTHER SUBSTANCES
Extracted: papaverine
Simultaneous: carbetapentane, cocaine, dioxyline, ethaverine, fluphenazine, imipramine, methapyrilene, papaveraldine, procaine, promethazine, strychnine, yohimbine
Interfering: diamorphine, thonzylamine

KEY WORDS
whole blood; pyrilamine (mepyramine) is IS

REFERENCE
Hoogewijs,G.; Michotte,Y.; Lambrecht,J.; Massart,D.L. High-performance liquid chromatographic determination of papaverine in whole blood, *J.Chromatogr.*, **1981**, *226*, 423–430.

SAMPLE
Matrix: formulations
Sample preparation: Crush 10 tablets, add 250 mL 50 mM HCl in EtOH:water 50:50, heat for 15 min on a steam bath, shake mechanically for 2 h, filter (glass fiber GF/A, Whatman), inject a 30 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil-10-ODS
Mobile phase: MeCN:buffer 50:50 (Buffer was 2.85 mM ethylenediamine sulfate adjusted to pH 7.44 ± 0.02 with 1 M ammonium hydroxide.)
Flow rate: 3.8
Injection volume: 30
Detector: UV 216.5